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oligonucleotide primer along said second polynucleotide is controlled relative to the extension of said oligonucleotide primer along said target sequence.

REMARKS

Reconsideration of this application in view of the present amendments and discussion is respectfully requested. A petition for extension of time for three months for responding to the outstanding Office Action.. Further enclosed is a Notice of Appeal to the Board of Patent Appeals and Interferences along with the requisite fee. Authorization to charge any appropriate fees is also enclosed.

The claims have been amended to more particularly define the present invention. Support for the amendments are found throughout the specification and drawings as filed. No new matter has been added by virtue of the claim amendments.

Claims 1-6 and 39-57 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

In formulating the rejection, the Office Action states on pages 2-3, bridging paragraph that :

> [T]he claim is drawn to an improved method of performing nucleic acid amplification reaction. The claim sets forth that one is to use a primer for performing primer extension reactions and the target nucleic acid can also be the result of a primer extension reaction. Taking applicant's argument at face value, it would appear that the primer ("primer A") which is used to perform a primer extension reaction can then be used as a template, or target nucleic acid for the very same reaction when a non-extended primer (primer A) is to be used. This cannot be. A primer will hybridize to a complementary sequence (non-primer A sequence), it will not hybridize to its own sequence (primer A).

It is believed that the rejection as to claims 1 to 6 has been addressed.

Applicant respectfully disagrees with the rejection as to claims 39-57. In particular, claim 39 (claims 40-57 dependent thereon) features a method for producing multiple copies of a target sequence where the primer used is hybridizable to the first and second flanking sequences of the target sequence. It would be readily apparent to those of skill that by hybridizing to both flanking sequences, the recited oligonucleotide primer is sufficient to amplify both target sequence strands e.g., as a stem-loop structure. See e.g., Applicant's specification at page 32, lines 1-8; page 36, lines 7-20; page 37, lines 1-3; page 59, line 7 to page 60, last line; and Figure 3.

Claims 4, 11, 27, 41 and 53 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The rejection is respectfully traversed.

In formulating the rejection, the Office Action states at page 3 that:

> Claims 4, 11, 27, 41, and 53 remain indefinite with respect to just what constitutes "substantially." In response to the above rejection, applicant has pointed to page 26, lines 13-20, of the specification as providing a definition of the term. Upon review of said page 26, not such definition can be located. It appears that page 26, rather than providing a definition to the above rejected term, provides a listing of organisms by genus species, starting with *Haemophilus aegypticus;* this listing of organisms extends from pages 24-30. Claims 5 and 6 which depend from claim 4; claims 12 and 13 which depend from claim 11; claims 42 and 43 which depend from claim 41; and claims 54-57 which depend from claim 53 fail to overcome this issue and are similarly indefinite.

The word "substantially" appears in the cited claims as "substantially identical". It is submitted that the phrase "substantially identical" is not vague or indefinite in view of the disclosure provided by Applicant's specification. For example, on page 46, lines 3-11, Applicant has specifically defined the phrase to mean two sequences which have at least 90% of the same or analogous base sequences where thymine (T) and uracil (U) are considered the same. That definition is believed to fully satisfy the requirements of §112, second paragraph. Withdrawal of the rejection is respectfully requested.

Claim 58 stands rejected under 35 U.S.C. §112, second paragraph as being indefinite.

In formulating the rejection, the Office Action states at page 3 that:

> Claim 58 is confusing with respect to the number and variety of primers. It is noted that the claim refers to "an oligonucleotide primer," yet it also states that "said primers are the same or different." If there are a plurality of primers, which are the one(s) that have the non-complementary 3'-end?

It is believed that the rejection has been addressed. Reconsideration and withdrawal of the rejection is requested.

Claims 1-58 stand rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent Nos. 4,683,202 (hereinafter "Mullis"); 5,391,480 (hereinafter "Davis") 5,618,664 (hereinafter "Kiessling"); 5,627,054 (hereinafter "Gillipsie"). The rejection is respectfully traversed.

In formulating this rejection, the Action states at pages 3 to 5 that:

> In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See In *re McLaughlin,* 443 F.2d 1392, 170 USPQ 209 (CCPA 1971).
>
> In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller,* 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). The test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference or that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art. *In re Keller,* 642 F.2d 413, 208 USPQ 871 (CCPA 1981j, at 208 USPQ 881. This is the standard employed herein.

> At page 8 of the response, it is asserted that the claimed method is "completely different" from that disclosed in the prior art and that "the primers of the present invention are essentially noncompetitive." Applicant's argument has been fully considered and has not been found persuasive for applicant is arguing limitations not present in the claims. Even if the claims were to be so amended, it is noted that the use of nested PCR (Mullis et al.), does not present a competitive primer sewing.
>
> At page 8 of the response it is asserted that none of the cited prior art teaches amplifying multiple templates in the same reaction. This assertion is wholy false for as pointed to in the prior Office action (see page 3, paragraph 5) Mullis et al., teach explicitly of performing nested PCR where two different sets of priers are used to amplify two different target sequences in the same reaction. As can be seen in Fig. 10, which represents the amplification product run on a gel, both sizes of anticipated amplification product are present (58-bp and 110-bp fragments). Clearly, the teachings of Mullis et al., and the types of labels that can be used, speak directly to quantitative and qualitative means for detection and are in direct opposition of applicant allegations at page 9 of the response that "Applicant can find no teaching or suggestion in the cited references of Applicant's method of providing qualitative or quantitative determination of multiple templates and of providing an indication of functional amplification conditions."

Before responding to the instant rejection, it is believed that a brief overview of Applicant's invention would be helpful.

The present invention addresses a long-felt need in the field of polymerase chain reaction (PCR) amplification. More specifically, the invention addresses recognized problems arising from replicating multiple polynucleotide templates in the same PCR reaction. For example, when it is useful to amplify a control template and a target template with the same primers in the same PCR reaction, there have been reports of undesirable competition between the templates. This competition can negatively impact outcome of the PCR, e.g., by lowering amplification efficiency and sensitivity. One solution to the problem has been to remove the control template, thereby essentially eliminating competition. However, that solution is not optimal in settings where presence of the control template is needed, e.g., to provide an indication of PCR reaction conditions.

The present invention solves this dilemma in several ways. For example, by providing ***controlled*** amplification of one template over the other, the invention enhances PCR efficiency and sensitivity. As is pointed out throughout Applicant's specification, that objective is achieved by using a specifically engineered primer with a 3'-mismatch (overhang) relative to its site of hybridization on one of the templates. The overhang is particularly configured to stall or prohibit replication on one of the templates, e.g., the control template, to which it is hybridized. Controlled amplification proceeds on those templates having complementarity at 3' end of the primer. Templates comprising primers with the overhang are not efficiently amplified.

The controllable amplification afforded by the present invention provides several advantages that are not taught or suggested by the combination of cited references. For example, as noted, contolled PCR amplification in accord with the invention can minimize and often eliminate competition between polynucleotide templates, thereby enhancing amplification efficiency and sensitivity. That advantage provides for more efficient quantitation of polynucleotide templates such as in settings involving infectious disease testing.

Additional advantages not taught or suggested by the combination of cited references are provided by the present invention. For example, the instant specification generally teaches amplification conditions that can regulate amplification of a desired template so that the amplification is more sensitive to the target. This feature of the invention positively impacts PCR, e.g., by allowing PCR amplification results to be more readily interpreted. That is, since amplification is controlled, PCR can proceed essentially to completion while preserving optimal sensitivity. In contrast, most prior PCR methods require quantitation during the exponential phase of the PCR. This feature of the invention is particularly important in settings where low levels of target need to be amplified and quantitated.

Additionally, the present invention is highly flexible and allows the user to manipulate a variety of PCR reaction conditions (e.g., template concentration) while still providing for controlled amplification of a desired template. For example, see Applicant's specification at Table 2, pg. 80 showing concentration ranges of template spanning about 6 logs.

Figure 1 of Applicant's disclosure shows one embodiment of the invention. For example, the figure shows amplification of a test polynucleotide (TPN) that is controlled relative to a control polynucleotide (CPN). In this particular embodiment, amplification of the TPN proceeds by extension of two primers (PP1, PP2) to produce extended templates (EPP1, EPP2). Amplification of the CPN is ***controlled*** relative to the TPN due to a 3' overhang sequence on PP1 that regulates both extension of PP1 and amplification of CPN. See also, pg. 30, line 22 to pg. 31, line 20; pg. 12 line 18 to pg. 13 line 11.

The presently claimed invention embodies methods for controllably amplifying templates. See claims 1 and 53 (featuring methods for controlling amplification of the second polynucleotide relative to the target); and claims 2, 9 25, and 39 (featuring amplification (extension) only after certain nucleotides are degraded).

Applicant's discovery of PCR amplification methods providing controlled amplification of one polynucleotide template over another template is an inventive concept not found in the cited references taken together or individually.

For example, neither Mullis (nor any of the other cited references) discloses a method for controlling PCR amplification on one polynucleotide template over another. In particular, Figure 10 (Example 10) of Mullis discussed in the Office Action shows amplification of ***one*** template type (homozygous or heterozygous betaglobin) per lane of a gel to give two amplification of *two* template products: a 58bp or a 110 bp DNA segment. Mullis' target sequences are believed to be on the same (one) template, not different templates. For example, according to Mullis at col. 26, lines 19-30 (emphasis added),

> This [Mullis'] procedure of using primers ***amplifying a smaller sequence contained within the sequence being amplified*** in the previous amplification process allows one to distinguish the wild-type from the sickle allele at the betaglobin locus ....

As understood, Mullis teaches amplification of a target sequence inside a sequence that is itself a target (ie. one template with multiple targets). Mullis, either taken alone or with the other cited references, provides no teaching, suggestion or motivation for controlling amplification of one template over another using a primer overhang as Applicant has done.

The disclosures of Gillepsie, Kiessling, and Davis fail to remedy this defect. Accordingly, reconsideration and withdrawal of the rejection is requested.

Applicant traverses the §103 rejection on additional grounds.

For example, the Office Action contends the Davis teaches use of a 3' to 5' exonuclease to remove a non-annealing terminal nucleotide. See Office Action dated September 30, 1997 at page 4. As understood, Davis' use of the exonuclease is to help remove a label from a template. In contrast to Davis, Applicant made the suprising observation that it was possible to use the 3' to 5' exonuclease (or polymerase having that activity) to cleave a primer overhang during PCR amplification. It is not obvious from Davis (or the other cited references) that the exonuclease (or polymerase) could remove the overhang consistently during multiple PCR cycles to facilitate efficient and controlled amplification. There is simply no teaching, suggestion or motivation in Davis or the other cited references to use the exonuclease or polymerase as Applicant has done. Accordingly, reconsideration and withdrawal of the rejection is requested.

Additionally, it is submitted that parts of the cited references have been overlooked by the Examiner. As an illustration, Gillepsie's primers are specifically taught in Gillepsie to be competitive. Thus, as mentioned in the previous response, it is believed that the Examiner's combination of cited references is a competitive amplification method. That method is completely different from the essentially non-competitive methods of the claims. Accordingly, the combination of cited references is not the Applicant's invention.

More particularly, it is believed that the instant rejection was formulated in large part by ignoring the competitive features of Gillepsie's primers while giving great weight to the apparent

inability of those primers to hybridize at a 3'-end (non-annealing). Where in any of the cited references, taken individually or in combination, is there a teaching, motivation or incentive for stripping specifically taught competitive features from Gillepsie's primers and using them as non-competitive primers? In contrast to the position taken by the rejection, the combination of cited patents is believed to be directed to a competitive amplification method. This is completely different from Applicant's invention. Accordingly, reconsideration and withdrawal of the rejection is requested.

The position has been taken that even if Applicant was to amend the claims to recite non-competitive amplification, "it is noted that the use of nested PCR (Mullis et al.), does not present a competitive primer setting". Office Action at page 5. Applicant believes that remark is not on point. As understood, the instant rejection was formulated to include use of Gillepsie's competitive primers, not any nested PCR primers reported by Mullis. See the Office Action dated September 17, 1997.

Accordingly, it is respectfully submitted that the Examiner has used hindsight reconstruction to formulate the instant rejection in view of Applicant's own disclosure.

For reasons already mentioned above, the kit of claims 53-57 are also unobvious in view of the Examiner's combination of references.

In view of the above discussion, it is respectfully submitted that the outstanding rejections have been overcome and should be withdrawn. Early reconsideration and notice of allowance are earnestly solicited.

Should the Examiner wish to discuss any of the amendments and/or remarks made

herein, the undersigned would appreciate the opportunity to do so.

Respectfully submitted,

Date: 9/30/98

Robert L. Buchanan (40,927)
Agent for Applicant
DIKE, BRONSTEIN, ROBERTS
& CUSHMAN, LLP
130 Water Street
Boston, Massachusetts 02109
(617-523-3400)